

(FILE 'HOME' ENTERED AT 07:41:49 ON 30 APR 2004)

FILE 'MEDLINE, CANCERLIT, EMBASE, BIOSIS, BIOTECHDS, CAPLUS' ENTERED AT  
07:42:10 ON 30 APR 2004

L1	72048 S FACTOR VIIA OR FACTOR VII OR FACTOR VIII OR FACTOR IX
L2	2382129 S PLASMID OR POLYMER OR MICROPARTICLE OR LIPID OR LIPOSOME OR C
L3	3170 S L1 AND L2
L4	148610 S GENE THERAPY
L5	522 S L4 AND L3
L6	428 DUP REM L5 (94 DUPLICATES REMOVED)
L7	4076186 S DOSE OR DOSAGE OR AMOUNT
L8	157 S L7 AND L6
L9	3453425 S REVIEW
L10	4 S L9 AND L6
L11	545823 S HEMOPHILIA OR COAGULATION OR BLEEDING
L12	220 S L11 AND L6
L13	56 S L12 AND L7
L14	52 S L13 AND PLASMID

=>

L10 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
 AN 2001-03389 BIOTECHDS  
 TI Gene transfer as an approach to treating hemophilia;  
 retro virus, **plasmid**, adeno-associated virus, adeno virus or  
 lenti virus vector-mediated **Factor-IX** gene  
 transfer and expression in skeletal muscle or liver for disease  
**gene therapy; a review**  
 AU High K A  
 CS Univ.Pennsylvania; Child.Hosp.Philadelphia  
 LO The Children's Hospital of Philadelphia, 3516 Civic Center Blvd, 310  
 Abramson Research Center, Philadelphia, PA 19104, USA.  
 Email: high@email.chop.edu  
 SO Circ.Res.; (2001) 88, 2, 137-44  
 CODEN: CIRUAL ISSN: 0009-7330  
 DT Journal  
 LA English  
 AB The use of gene transfer as an approach to treating hemophilia is  
 reviewed. Present treatment for hemophilia involves i.v. infusion of  
 either recombinant or plasma-derived clotting factor concentrates.  
 However, this method of treatment is expensive and there are risks of  
 blood-borne disease transmission. Hemophilia has a number of advantages  
 as a model system for working out strategies for gene transfer as an  
 approach to the **gene therapy** of genetic diseases,  
 which include: wide latitude in choice of target tissue; broad  
 therapeutic window for levels of circulating factor; ease of determining  
 therapeutic endpoints; and existence of excellent animal models of the  
 disease. Three clinical trials, each using different vectors and target  
 tissues, are currently in progress, and 2 additional trials are in late  
 planning stages. Strategies of **gene therapy** and gene  
 transfer for hemophilia therapy have included using: retro virus-mediated  
 approaches; **plasmid**-based approaches; adeno-associated virus  
 vector approaches expressing **Factor-IX** to skeletal  
 muscle or the liver; adeno virus-mediated approaches; and lenti  
 virus-mediated approaches. (70 ref)

L14 ANSWER 42 OF 52 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
AN 2003-07322 BIOTECHDS  
TI New non-viral vesicle vector comprises vesicular membrane with hepatitis  
B envelope protein and nucleic acid expression construct comprising  
complete **factor VIII** or **IX** coding sequence, useful  
for treating **hemophilia**;  
vector-mediated gene transfer and expression in host cell useful for  
**hemophilia gene therapy**

AU CHIEN K R; HOSHIJIMA M  
PA UNIV CALIFORNIA  
PI WO 2002086091 31 Oct 2002  
AI WO 2002-US13164 25 Apr 2002  
PRAI US 2001-286314 25 Apr 2001; US 2001-286314 25 Apr 2001  
DT Patent  
LA English  
OS WPI: 2003-093125 [08]  
AB DERWENT ABSTRACT:  
NOVELTY - Non-viral vesicle vector comprising: (a) a vesicular membrane  
with hepatitis B envelope protein exposed on the vesicle surface; or (b)  
a nucleic acid expression construct comprising a complete **factor**  
**VIII** or **factor IX** coding sequence and a  
promoter sequence functional in liver cells, is new.  
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for  
treating **hemophilia**.  
BIOTECHNOLOGY - Preferred Vector: The envelope protein of the  
non-viral vesicle vector contains mutations to reduce antigenicity. The  
expression construct is double stranded **plasmid** DNA or RNA and  
comprises inverted terminal repeat sequences from adeno-associated virus  
(AAV-ITR), eukaryotic transposon, transposase sequences, the coding  
sequence of **factor VIII** or **IX**. The **factor**  
**VIII** comprises silent mutations to enhance expression. The  
promoter is a non-tissue specific promoter comprising cytomegalovirus,  
Rous sarcoma virus, ubiquitin, chicken beta-actin or elongation factor  
1alpha promoter, or preferably liver specific promoter. The liver  
specific promoter comprises alpha-fetoprotein promoter, globulin  
promoter, approximately 1-microglobulin or albumin. Preferred Method:  
Treating **hemophilia** comprises: (a) administering into  
circulation of an individual with **hemophilia** the non-viral  
vesicle vector and the nucleic acid expression construct; and (b)  
monitoring the individual for amelioration of disease. Administration  
into circulation comprises intravenous or intraarterial administration,  
particularly into hepatic or portal artery.  
ACTIVITY - Hemostatic. No suitable data given.  
MECHANISM OF ACTION - **Gene therapy**.  
USE - The non-viral vesicle vector is useful for treating  
**hemophilia** (claimed).  
ADMINISTRATION - The non-viral vesicle vector and the nucleic acid  
expression construct is administered via intravenous or intraarterial  
route, particularly into hepatic or portal artery (claimed). No  
dosage given. (34 pages)

L14 ANSWER 4 OF 52 MEDLINE on STN  
 AN 2001231742 MEDLINE  
 DN PubMed ID: 11319920  
 TI **Gene therapy** for the treatment of **hemophilia**  
 B using PINC-formulated **plasmid** delivered to muscle with  
 electroporation.  
 AU Fewell J G; MacLaughlin F; Mehta V; Gondo M; Nicol F; Wilson E; Smith L C  
 CS Valentis, Inc., The Woodlands, Texas 77381, USA.  
 SO Molecular therapy : journal of the American Society of Gene Therapy, (2001  
 Apr) 3 (4) 574-83.  
 Journal code: 100890581. ISSN: 1525-0016.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 200107  
 ED Entered STN: 20010730  
 Last Updated on STN: 20010730  
 Entered Medline: 20010726  
 AB **Gene therapy**, as a safe and efficacious treatment or  
 prevention of diseases, is one of the next fundamental medical  
 innovations. Direct injection of **plasmid** into skeletal muscle  
 is still a relatively inefficient and highly variable method of gene  
 transfer. However, published reports have shown that application of an  
 electric field to the muscle immediately after **plasmid** injection  
 increases gene expression at least 2 orders of magnitude. Using this  
 methodology, we have achieved potentially therapeutic circulating levels  
 of human **factor IX** (hF.IX) in mice and dogs. A  
**plasmid** encoding hF.IX formulated with a protective, interactive,  
 noncondensing (PINC) **polymer** was injected into the skeletal  
 muscle followed by administration of multiple electrical pulses  
 (electroporation). In mice long-term expression was achieved and the  
 ability to readminister formulated **plasmid** was demonstrated. In  
 normal dogs, expression of hF.IX reached 0.5-1.0% of normal levels. The  
 transient response in dogs was due to the development of antibodies  
 against hF.IX. Elevated circulating creatine kinase levels and  
 histological examination indicated transient minor trauma associated with  
 the procedure. These data show that gene delivery using a **plasmid**  
 formulated with a PINC **polymer** augmented with electroporation is  
 scalable into large animal models and represents a promising approach for  
 treating patients with **hemophilia B**.

L14 ANSWER 3 OF 52 MEDLINE on STN  
 AN 2001253005 MEDLINE  
 DN PubMed ID: 11273783  
 TI Linear DNAs concatemerize in vivo and result in sustained transgene expression in mouse liver.  
 AU Chen Z Y; Yant S R; He C Y; Meuse L; Shen S; Kay M A  
 CS Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305, USA.  
 NC DK49022 (NIDDK)  
 SO Molecular therapy : journal of the American Society of Gene Therapy, (2001 Mar) 3 (3) 403-10.  
 Journal code: 100890581. ISSN: 1525-0016.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 200108  
 ED Entered STN: 20010806  
 Last Updated on STN: 20010806  
 Entered Medline: 20010802  
 AB The short duration of transgene expression remains a major obstacle for the implementation of nonviral DNA vectors in clinical **gene therapy** trials. Here, we demonstrate stable, long-term transgene expression in vivo by transfecting a linear DNA expression cassette (LDNA) into mouse liver. Interestingly, despite similar quantities and cellular distribution of injected DNAs in their livers, mice receiving LDNA encoding human alpha1-antitrypsin (hAAT) expressed approximately 10- to 100-fold more serum hAAT than mice injected with closed circular (cc) DNA for a period of 9 months (length of study). Furthermore, when a linear human **factor IX** expression cassette was delivered to **factor IX**-deficient mice, sustained serum concentrations of more than 4 microg/ml (80% of normal) of the human clotting factor and correction of the **bleeding** diathesis were obtained. Southern blot analyses indicate that, unlike ccDNA, LDNA rapidly formed large, unintegrated concatemers in vivo, suggesting that transgene persistence from **plasmid**-based vectors was influenced by the structure of the vector in transfected cells. No differences in transgene expression or DNA molecular structures were observed when AAV ITRs were included to flank the hAAT expression cassette in both ccDNA- and LDNA-treated animals. Linear DNA transfection provides an approach for achieving long-term expression of a transgene in vivo.

L14 ANSWER 2 OF 52 MEDLINE on STN  
 AN 2001345700 MEDLINE  
 DN PubMed ID: 11407909  
 TI Long-term and therapeutic-level hepatic gene expression of human  
**factor IX** after naked **plasmid** transfer in  
 vivo.  
 AU Miao C H; Thompson A R; Loeb K; Ye X  
 CS Puget Sound Blood Center, University of Washington, Seattle, Washington  
 98104, USA.. miao@u.washington.edu  
 SO Molecular therapy : journal of the American Society of Gene Therapy, (2001  
 Jun) 3 (6) 947-57.  
 Journal code: 100890581. ISSN: 1525-0016.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 200108  
 ED Entered STN: 20010903  
 Last Updated on STN: 20010903  
 Entered Medline: 20010830  
 AB Naked DNA transfer of a high-expressing human **factor IX**  
 (hFIX) **plasmid** yielded long-term (over 1 1/2 years) and  
 therapeutic-level (0.5-2 microg/ml) gene expression of hFIX from mouse  
 livers. The expression cassette contained a hepatic locus control region  
 from the ApoE gene locus, an alpha1-anti-trypsin promoter, hFIX cDNA, a  
 portion of the hFIX first intron, and a bovine growth hormone  
 polyadenylation signal. In contrast, a hFIX **plasmid** containing  
 the expression cassette without effective regulatory elements produced  
 initially low-level gene expression that rapidly declined to undetectable  
 levels. Southern analyses of the cellular DNA indicated that the majority  
 of the input genome from either vector persisted as episomal forms of the  
 original plasmids. Together with RT-PCR analyses of the transcripts,  
 these data indicated that at least two processes are critical for  
 sustained gene expression: persistence of vector DNA and  
 transcriptional/posttranscriptional activation. Liver regeneration after  
 partial hepatectomy resulted in a significant decline in transgene  
 expression, further suggestive of decreased episomal **plasmid**  
 maintenance rather than transgene integration. Transaminase levels and  
 liver histology showed that rapid intravenous **plasmid** injection  
 into mice induced transient focal acute liver damage (< 5% of  
 hepatocytes), which was rapidly repaired within 3 to 10 days and resulted  
 thereafter in histologically normal tissue. No significant differences  
 were observed between rapid injection of **plasmid** and saline  
 control solutions. Transient, very low level antibodies directed against  
 hFIX did not prevent the circulation of therapeutic levels of the protein.  
 Gene transfer of hFIX **plasmid** DNA into liver elicited neither  
 transgene-specific cytotoxic effect nor long-term toxicity. These results  
 demonstrate that long-term expression of hFIX can be achieved by nonviral  
**plasmid** transfer and suggest that this occurs independent of  
 integration.

L14 ANSWER 6 OF 52 MEDLINE on STN

AN 95394346 MEDLINE

DN PubMed ID: 7665069

TI **Liposome**-encapsulated DNA-mediated gene transfer and synthesis of human **factor IX** in mice.

AU Baru M; Axelrod J H; Nur I

CS Octa Medical Research Institute, Kiryat Weizmann, Rehovot, Israel.

SO Gene, (1995 Aug 19) 161 (2) 143-50.

Journal code: 7706761. ISSN: 0378-1119.

CY Netherlands

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199510

ED Entered STN: 19951020

Last Updated on STN: 19990129

Entered Medline: 19951006

AB **Hemophilia B** is an X-chromosome-linked recessive disorder that is caused by a deficiency of biologically active clotting **factor IX** (FIX). In this work, liposomes (Lip) were used for non-viral, in vivo gene transfer of the human FIX gene into mouse organs. **Plasmid** DNA, containing the human FIX cDNA under the control of the Moloney murine leukemia virus (MoMLV) long terminal repeat (LTR), was encapsulated in 1-2-microns multilamellar Lip composed of egg phosphatidylcholine (EPC). The percentage of Lip-associated DNA was 47%, and 72% of the Lip DNA was protected from DNase I digestion. The Lip-encapsulated (Len) DNA was injected intravenously into Balb/c mice, and at various times post-injection, various tissues were examined for the presence of the exogenous DNA. **Plasmid** DNA was detected by Southern blot analysis mainly in the liver and spleen, but small amounts were also detected in the lungs, heart and kidneys. The **plasmid** DNA was retained in mouse liver cells for at least 7 days post-injection, and remained in an episomal state. The levels of human FIX protein in the mouse plasma were 190-650 pg per ml for 2 to 7 days post-injection. Treatment of mice with chloroquine (Cq) and colchicine (Cc) prior to Lip injection significantly increased the **amount of plasmid** DNA found in the liver cells, as well as the level of human FIX in the plasma. These results demonstrate the potential use of Len DNA for gene transfer into liver and spleen, and for **gene therapy** of inherited and acquired disorders.